CHAPTER 6

Identifying and Characterizing Reference Conditions

- A. Significance of Reference Conditions
- B. Approaches to Establishing Reference Conditions
- C. Initial Data Retrieval and Processing to Support Nutrient Criteria Development

A. Significance of Reference Conditions

Establishing lake reference conditions for each of the physical lake classes within each ecoregion or subdivision of that region is a critical part of the nutrient criteria development process. Reference conditions for each of these classes are the quantitative descriptions of lake conditions used as a standard for comparison purposes. Ideally, reference conditions associated with nutrient-related variables such as phosphorus, nitrogen, and chlorophyll *a* are concentrations representative of lake conditions in the absence of anthropogenic disturbances and pollution. However, because it can be argued that most, if not all, lakes have been impacted by human activity to some degree, reference conditions realistically represent the least impacted conditions or what is considered to be the most attainable conditions. While the reference conditions themselves are not specifically established as criteria, they help to set the upper bounds of what can be considered the most natural and attainable lake conditions for a specific region. Knowing this "reference best case situation" together with the other four elements of a nutrient criterion allows resource managers to set the criteria at appropriate levels; of course, other relevant factors such as attainment-of-use designation, regional perceptions of water quality, and physical/geological influences need to be considered as well.

B. Approaches to Establishing Reference Conditions

In accordance with the five elements of nutrient criteria development, there are three general approaches for establishing reference conditions:

- Direct observation (data collection) of sites and estimation or inference of reference conditions. This may take two forms: (1) observation of sites that meet reference site requirements and (2) observation (data) of an entire population of lakes. It is assumed that some percentile of either distribution represents a reference condition.
- Paleolimnological reconstruction of past conditions. This means inference of reference conditions from observations of nonreference sites. It requires statistical models based on large data sets and a sample of dated sediment cores for the lake classes in question.
- Model-based prediction or extrapolation of reference conditions from related data sets or related knowledge. The predictions may come from statistical models (usually regression models), mass balance models, or combinations of the two.

These three approaches are not exclusive, and they require professional judgment and expertise to implement. Regional experts are often the most qualified to make determinations about which lakes in a region most likely represent "ideal" or most desirable conditions. Local expert knowledge of regional

characteristics, human-induced changes over time to regional lake and watershed conditions, patterns of development associated with a lake and its watershed, and current conditions as they relate to perceived impaired or unimpaired status can many times be the most efficient and cost-effective way of identifying candidate reference lakes. This method should be used only when it is clear that adequate professional expertise exists for a region. Experts who may be qualified to participate in the selection process could come from an array of disciplines such as limnology, biology, resource management, engineering, and other related fields.

1. Direct Observation of Reference Lakes

■ Reference Lake Approach

Reference lakes must be representative of a region and their conditions (including level of enrichment that may inherently range from oligotrophic to eutrophic or hypereutrophic) should represent the best range of minimally impacted conditions that can be expected of similar lakes within the region. Although lakes that are undisturbed by human activities are ideal as reference sites, land use practices and atmospheric pollution have so altered the landscape and quality of water resources nationally that truly undisturbed lakes are rarely available.

A set of requirements may be established to help define a minimally impacted lake and, therefore, make the selection of reference sites a rule-based procedure. For example, reference lakes could be chosen only from park or preserve areas (i.e., areas that have not been subjected to any type of significant development within a reasonable period of time). If relatively unimpacted conditions do not occur in the region, the selection process may be modified to be more realistic and to reflect the least altered lakes. Such selection can pertain to the condition of the watershed, as well as the lake itself. The following are examples of conditions that can be defined to select reference lakes:

- Land use. Natural vegetation has a positive effect on water quality and hydrological response of streams. Reference lakes could be chosen from watersheds that have an established minimum percentage of existing natural vegetation cover, such as 50 percent or more. Alternatively, reference criteria could be defined as less than a certain percentage of urban or residential land use in the watershed.
- Riparian zones. Zones of natural vegetation alongside the lakeshore and streams stabilize shorelines from erosion and contribute to the aquatic food source through allochthonous input. They also reduce nonpoint pollution by absorbing and neutralizing nutrients and contaminants. Reference lakes could be chosen from watersheds that contain at least a set minimum area of existing natural riparian zone, regardless of land use outside the riparian buffers. For example, reference lakes could be required to have 65 percent or more of the lakeshore and its immediate tributaries in natural bankside vegetation to a distance of at least 10 m from the shoreline.
- Best management practices. Urban, industrial, suburban, and agricultural nonpoint source pollution can be reduced with successful best management practices (BMPs). Watersheds from which reference lakes are chosen could be required to have certain BMPs in place, provided that the efficacy of the BMPs has been demonstrated.

- *Discharges*. Point source discharges from industry and from municipal waste water treatment plants (e.g., National Pollutant Discharge Elimination System, storm water) and known areas of nonpoint source discharges (e.g., from agricultural areas) have the potential to negatively affect water quality of streams, rivers, and lakes. Reference lakes could be chosen from watersheds that have no discharges or that have a defined maximum level of discharges into surface waters.
- Management. Management actions, such as controlling water level fluctuations for hydropower
 or flood control, can significantly influence lake conditions. Reference lakes could be limited to
 those lakes that are in no way affected or are affected only in a very limited way by such
 management activities.

Predefined reference conditions for lakes have been used in Minnesota to determine ambient phosphorus criteria (Heiskary, 1989) (see case study at the end of this chapter). Maine uses a similar approach in regulating the water quality of streams (Davies et al., 1993).

Characterizing Reference Conditions

The objective of reference condition characterization is to describe reference lake conditions in terms of the variables that have been chosen to express nutrient conditions. To properly characterize reference conditions, adequate data associated with the reference lakes must be available. If existing data are deemed to be insufficient for characterization purposes (see Chapter 4), then a sufficient number of reference lakes must be sampled to obtain the data necessary for characterization of reference conditions. There is no definitive rule for establishing a minimum number of reference sites appropriate to the development of a reference condition, and the greater number the better. The amount of available data will dictate where each lake is a sampling unit—perhaps a general rule of thumb for reference sample size could be at least 10 percent of the lake class of concern and a minimum of 10 to 30 lakes per region (see Chapter 4 for a discussion of sampling approaches and methodologies). Before sampling begins, candidate variables should be evaluated and target variables selected (see Chapter 5). All sampling and analytical activities should be conducted according to standard protocols to ensure validity.

During sampling visits, the candidate reference lakes should be examined to confirm whether they actually meet the reference site requirements. This may include looking for discharges into the lake or its tributaries and a quick survey of the lake watershed to determine if new modifications may have changed the lake. If a lake appears to have been significantly impacted, measurements should be made for in-lake and lake discharge nutrient concentrations and biological response variables such as chlorophyll concentration and fish, macroinvertebrate, macrophyte, and planktonic community variables. Lakes that do not meet the physical reference requirements should be excluded from the reference data set. However, a high nutrient concentration present in an otherwise minimally developed lake watershed is not justification alone for exclusion. This may be part of the natural background level to be identified by the reference condition process.

Significantly Altered Lakes

If all lakes in a region are significantly altered, it might not be possible to select appropriate reference sites. In such a case, an alternative would be to use lakes from neighboring regions as reference sites if those lakes are deemed acceptable, by professional judgment, with respect to impact and overall comparability to the lakes of the affected region. If lakes from nearby regions cannot reasonably be considered reference sites, then reference lakes must be identified and reference conditions must be predicted or inferred from other information, including models and historical data. In designing such an approach, the consensus of a panel of regional experts helps ensure an objective and rational design.

Once the desired lake reference data have been obtained, statistical approaches can be used to determine if individual lakes fit the preliminary reference lake classification. The preliminary classification is refined through inspection of plotted data (e.g., using box and whisker plots, scatter plots, or other means of graphical analysis), professional judgment, and statistical tests of final classification hypotheses. Next, the values and distribution of reference metrics are compared among ecoregion or lake type. Regions that appear to be similar to each other can be combined for the final classification. For two regions to be so combined, most of the metric distributions must be similar. Chapter 7 further describes the derivation of criteria from these reference lake data sets. States that share an ecoregion are encouraged to also share data to determine reference conditions.

■ Lake Population Distribution Approach

If a fixed definition of reference condition is deemed to be overly restrictive or an impractical ideal, then an empirical working definition is an alternative. For example, because natural conditions for reservoirs cannot be defined, the best existing conditions are used instead. This approach is also useful in ecoregions with little contiguous natural vegetation remaining, such as in the agricultural Midwest. The approach does not involve the identification of reference lakes, but sets reference conditions by using an entire population of lakes from within a region. It is especially relevant for man-made impoundments and reservoirs, where no least-impaired systems exist, as well as for lakes subject to strong and relatively uniform human impacts, such as in large urbanized areas or in heavily agricultural regions.

A representative sample of lakes is taken from the entire regional lake population. Lakes that are known to be severely impaired may be excluded from the sample, if desired. The population distribution of each selected variable is determined, and the best quartile or lower 25th percentile of the distribution of each variable is taken as its reference value. Using the same logic described for Secchi depth, the opposite end of the distribution is used, and the quartile at the high-quality end of this distribution is selected so this reference value is a "reasonable" upper limit (excluding outliers of the population distribution). See Chapter 7 for further discussion.

A central assumption of the frequency distribution approach is that at least some sites in the population of lakes are high-quality lakes, which will be reflected in the values of the individual variables. Because they have no independent definition, reference conditions defined in this way must be taken as preliminary and subject to future reinterpretation, especially as lake management efforts produce improved conditions. Periodic examination of the reference values for trends can detect deterioration or improvement. This is necessary when reference criteria cannot be defined a priori or when all lakes under consideration are equally impaired. The objective of the method is to develop a measurement standard for assessing lakes. Its validity is supported by external confirmation of the response of variables to stressors, usually from published or other independent studies. While direct observation of reference lakes is the preferred approach, paleolimnological reconstruction and model extrapolation are also possible approaches for reference condition determination.

2. Paleolimnological Reconstruction

Many groups of organisms in lakes leave remains in the bottom sediments. Some of the remains are resistant to decay and become a permanent biological record of life in the lake. By comparing past biota with present-day biota of many lakes, past environmental conditions can be inferred. Several groups of organisms have been used for paleolimnology: diatoms and chrysophytes, sponges, bryozoans, cladocerans, and chironomid larvae. Of these, diatom frustules and chrysophyte scales have been used most often, and most successfully, to infer past chemical conditions (e.g., Charles and Smol, 1994). The

preserved diatoms provide an integrated record of the diatom assemblage in the lake. A sample of the top 1 to 2 cm of lake sediment contains a representative sample of diatoms from the most recent 1 to 3 years. If the lake sediments remain undisturbed, then diatoms and chrysophytes preserved in lake sediments are integrators of lake history (Charles et al., 1994; Dixit et al., 1992).

Inference of past nutrient conditions from biological remains is based on strong relationships of biota with water quality. Many algal species are indicators of particular nutrient conditions, and the assemblage found can therefore be used to infer nutrient conditions. Environmental variables such as alkalinity, aluminum, dissolved organic carbon, salinity, nickel, conductivity, calcium, TN, TP, Secchi transparency, and trophic state also have been inferred using diatom-based predictive models (Charles et al., 1994; Dixit et al., 1992; Fritz, 1990). Sediment cores can be calibrated with other information (e.g., varves, known contamination events, radioisotopes, pollen) to obtain time series with resolution of up to 1 to 10 years. Therefore, the diatom and chrysophyte fossil record can also aid in establishing reference conditions by enhancing the historical record of previous data collections and events.

Paleolimnological analysis, part of the Environmental Monitoring and Assessment Program protocol (U.S. EPA, 1994b), requires development of a data set that associates current environmental conditions with current surficial diatom assemblages. Present-day associations are used to infer past conditions based on fossil diatom assemblages in deeper sediment layers. Quantitative prediction is usually done in two steps: (1) development of predictive models (calibration or transfer functions) followed by (2) use of the models to infer environmental variables from fossil assemblages (Charles and Smol, 1994).

■ Inference of Past Conditions

Inference of past environmental conditions from fossil assemblages requires a sizable calibration data set that includes both assemblages and environmental (chemical) measurements. The calibration data set typically consists of recent sediment samples (top 1 cm) with complete lake water quality and chemical estimation from at least 50 lakes. The calibration data set should include lakes that span the ranges of all important environmental variables that are being investigated. For example, development of predictive models for acidification requires a calibration data set that includes alkaline, neutral, and acidic lakes; development of predictive models for nutrient concentrations requires a calibration data set that includes lakes with high and low concentrations of nitrogen, phosphorus, and silica and combinations of the nutrients. The sedimented diatom assemblage is identified and enumerated for each lake in the calibration sample.

The first step is to determine whether species-environment relationships are strong enough to permit development of predictive models for inference of environmental conditions. Currently, this is done using canonical correspondence analysis (CCA) (Dixit et al., 1992; Jongman et al., 1987; ter Braak, 1986). CCA is an ordination technique that orders sites on environmental gradients. Unlike linear models such as canonical correlation, there is no assumption that species abundance is a linear response to environmental gradients. Instead, species are assumed to have a unimodal response to gradients, such that abundance of a species is reduced above and below the optimum value of an environmental variable (ter Braak, 1986). The assumption of modal responses, where each species has optimum values of nutrients, temperature, light, pH, etc., is more realistic for algae than are linear responses (e.g., Tilman, 1982). The CCA identifies environmental variables that have strong associations with species composition and therefore are suitable for predictive model development.

Models to predict values of environmental variables (e.g., nutrients) are developed with weighted averaging regression (Charles and Smol, 1994). The computer program WACALIB (Line et al., 1994) is

an efficient way to perform these calculations. The technique also uses the assumption of unimodal optima of environmental variables, as does CCA. The optimum condition for each taxon is the average of mean values for the environmental variable at sites in which the taxon is found, weighted by the abundance of the taxon (Charles and Smol, 1994). The inferred value of the environmental variable is in turn the sum of optima (for that variable) for all taxa at a site, weighted by the relative abundance of each taxon. Techniques have also been developed to quantify error and uncertainty of the predictions (Birks et al., 1990). Further documentation of these methods is in Charles and Smol (1994), Charles et al. (1994), Birks et al. (1990), and ter Braak and Juggins (1993).

■ Existing Diatom Databases

Paleolimnological databases exist for several regions of the country. The largest of these is the Paleoecological Investigation of Recent Lake Acidification, which is for the Northeast, but other databases exist for the Upper Midwest and Florida. Paleolimnology to establish reference conditions for impoundments and reservoirs is not recommended because impoundments undergo succession of several years or more following inundation. A reference as an attainable condition thus would be impossible to define for reservoirs from paleolimnological data.

3. Model Prediction and Extrapolation

If few or no limnological data are available for a given lake or region, reference conditions can be predicted by inference or extrapolation of various models. Although extrapolation of models beyond their original calibration data is risky, it may be the only option to estimate reference conditions if no reference data exist or are likely to exist. There are two approaches: (1) using the morphoedaphic index method (MEI) and (2) extrapolating natural background nutrient loading that would occur under undisturbed conditions followed by estimation of nutrient concentrations and trophic state with a mass balance model. These methods are discussed in more detail in Chapter 8.

The MEI is the ratio of total dissolved solids in lake water to the mean depth of the lake. Several early studies suggested that the MEI was correlated with fish and phytoplankton production of lakes (e.g., Rawson, 1951; Ryder, 1961; Oglesby, 1977). The MEI approach was extended by Vighi and Chiaudani (1985) to predict phosphorus concentrations resulting from natural background loading in undisturbed watersheds—in short, to predict reference phosphorus concentrations. The MEI approach is simple and appears to be highly successful for a limited set of cool-temperate lakes. It has been largely ignored by North American limnologists, possibly because of its simplicity, and as a result, it has not been calibrated and tested for a wider variety of lakes. The approach is promising, but it needs to be recalibrated and tested with regional reference lake data sets.

Mass balance models are a means of estimating concentrations of substances (primarily nutrients) from knowledge of the loading into a lake and the hydrology of a lake (see Chapter 2, Section C). A mass balance model by itself will not establish reference conditions, but it will predict nutrient concentrations given certain loading values. Therefore, use of a mass balance model to derive reference conditions requires an estimate of natural background nutrient loading to a lake.

If a lake or lakes of a region are primarily stream-fed, an estimate of background nutrient loading may be made from stream water quality data with some method for selecting (or assuming) a reference distribution or reference value of stream nutrient concentrations.

Fulmer and Cooke (1990) used a frequency distribution approach combined with loading and mass balance models to estimate the reference conditions of a State's water bodies. They estimated the phosphorus concentration in 19 Ohio reservoirs based on the characteristics in the ecoregions in which the reservoirs were located. For the incoming phosphorus concentration, they used the 25th percentile of stream phosphorus concentration in four of Ohio's ecoregions (the best quartile). They termed the resulting phosphorus estimate the "attainable phosphorus concentration," suggesting that this was a conservative estimate of the stream phosphorus concentration attainable through waste water treatment, improvements in agricultural practices, and treatment of urban runoff in the watershed.

The authors then compared this estimated phosphorus value to that actually found in the reservoir after first transforming the values to the trophic state index values of Carlson (1977). The difference between the trophic state indices was termed "the restoration potential" of the water body. This would target those lakes that deviate the most from the attainable trophic state for that body. This approach is important because it combines the use of the ecoregion to estimate reference conditions for the reservoir with the emphasis of differing restoration potentials based on the specific characteristics of the individual reservoir. This simple but effective approach applies only to stream-fed lakes (principally reservoirs). Ground-water-fed and head water lakes would require one of the other methods for estimating loading that does not rely on stream concentrations.

Many states have ongoing biological criteria programs and have identified least stressed streams as reference sites. These biological reference sites could likewise serve as reference sites for stream nutrient criteria and background nutrient concentrations and loading. This method would be appropriate only for impoundments and flowage lakes, which are stream-fed, and not for lakes with substantial ground water input.

C. Initial Data Retrieval and Processing to Support Ecoregional Nutrient Criteria Development

Nutrient data will be collected for site points (years 1990 to present) from STORET, the U.S. Geological Survey's NAWQA, and other acceptable databases and then will be grouped by nutrient ecoregion (Omernik's Level III aggregations). The headquarters nutrient team and Regional Technical Assistance Groups (RTAGs) will assess the data and will determine regional reference condition values for TP, TN, chlorophyll *a*, and Secchi depth.

A regional reference condition can be selected using either of two approaches. In both instances, the intention is to select an optimal reference condition value from the distribution of an available set of lake data for a given physical class of lakes or reservoirs.

One approach is to select a percentile from the distribution of measured variables of known reference lakes (e.g., highest quality or least impacted lakes of that size class of all lakes in the region). The variables of initial concern are TP, TN, chlorophyll *a*, and Secchi depth. Because these reference lakes are already acknowledged to be in an approximately ideal state, it is reasonable to select an upper percentile (i.e., a representation of the lesser nutrient quality of the distribution of the reference lakes) as the reference condition. EPA generally recommends the upper 25th percentile (Figure 6.1(a)).

The other approach is to select a percentile from all lakes in the class or from a random sample distribution of all lakes in the class. In this case, it should be a lower percentile (i.e., a representation of the better end of the range of all lakes) because the sample is expected to contain at least some degraded lakes if it is an entire population or a truly random selection. This option is most useful in regions where the number of legitimate natural reference water bodies is usually very small such as highly developed

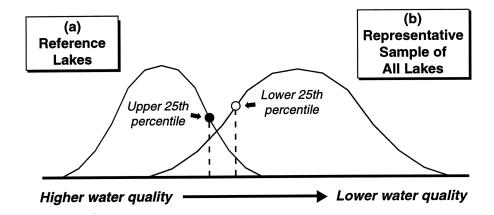


Figure 6.1. Two approaches for establishing a reference condition value. Note: Percentiles are based on order statistics, statistics derived from ordering data from low to high or high to low. In the case of TP, TN, and chlorophyll *a*, higher concentrations of the variable result in lower water quality. Consequently, the scale presented above is ordered from low to high. A similar analysis of Secchi depth, however, would require ordering the data from high to low because higher Secchi disk readings are associated with higher water quality.

land use areas like the agricultural lands of the Midwest and the urbanized east or west coasts. EPA's recommendation in this case is usually the lower 25th percentile, depending on the number of natural reference lakes available (Figure 6.1(b)). If almost all reference lakes are impacted to some extent, then the lower 5th percentile might be used in an effort to approximate previous natural conditions.

It should be noted that the upper 25th percentile of a reference lake distribution and the lower 25th percentile from a representative sample distribution are general recommendations. The actual distribution of the observations and knowledge of the inherent regional water quality are also determinants of the threshold point chosen.

In Figure 6.2, TP is the variable of concern and illustrates the presumption that, all else being equal, these two alternative methods should approach a common reference condition along a continuum of data points. In this figure, the upper 25th percentile of the collection of reference lakes produces a TP reference condition of 30 μ g/L. The lower 25th percentile of the random sample of lakes produces a value of 35 μ g/L. The shaded portions of both distributions in Figure 6.2 indicate those lakes in each sample distribution that presumably would not meet reference expectations. It should be noted that (1) variability about these selected points is a factor in any such estimation, (2) this is a reference condition value subject to further evaluation before a nutrient criterion is developed, and (3) given that overenrichment is a recognized water quality problem, it must be expected that some fraction of the present measured water bodies would be so identified.

Because there is little distinction in this case, the Agency may choose to select either $30 \,\mu g/L$, $35 \,\mu g/L$, or the intermediate $33 \,\mu g/L$ as the TP reference condition value. Each State or Tribe should similarly calculate its reference condition using both approaches to first determine which method is most protective. Then they should use that most protective approach for their subsequent reference condition calculations.

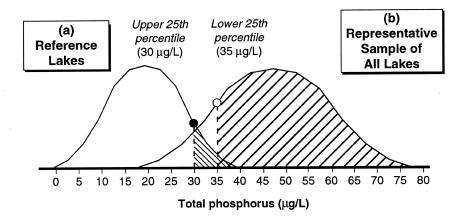


Figure 6.2. Two approaches for establishing a reference condition value using total phosphorus as the example.

In most instances, EPA will calculate regional nutrient criteria reference conditions by first subdividing the data set into size classes of lakes. These subdivided data then will be assessed to determine if further subdivision of the regional boundaries is necessary. If data tend to cluster within a nutrient ecoregion, it may indicate the need for further refinement of those regional boundaries into subregions.

Once the regional boundaries are determined, a frequency distribution selection for each lake class per region or subregion is established at the lower 25th percentile. This is because the available data cannot usually be assured of being all reference condition quality. The data are obtained from a wide variety of sources with an equally wide array of documentation and quality assurance procedures. The Agency policy is to choose consistently lower end percentiles to assure the appropriate protection of the uses. This conservative effort, together with consulting the RTAGs in determining these regional criteria, is designed to help protect against setting the regional criteria either too high or too low.

Another element that must be considered in setting the reference conditions is where and when the data were gathered. If the sample size is large enough, the time of year the individual samples were taken may not matter; either all seasons will be represented or most of the data will cluster about an appropriate index season. Similarly, surface grab or depth-selected samples or composite samples may not matter if the diverse data set is large enough. However, when these factors are significant, the most indicative time of the year should be used as the index period. Alternatively, criteria should be developed for each season of the year. Likewise, the depth and location of sampling in the lakes in question that best reveals the presence and amount of nutrients in that system should be used. These are usually within the upper meter of surface water from a central portion of the water body or open water area near the main discharge location. This guideline applies to measurement of both causal and response variables. Once again, it is preferable to err on the side of the environment.

Once the reference condition is determined, the other four elements of criteria development should be incorporated in the process. First, the reference condition should be compared with the historical information for that area. Are things getting generally better or worse than before? Model predictions not only fill the void when there is a lack of sufficient reference condition data, but they may help answer this

trend question. All of this accumulated diverse information requires careful and objective review by the RTAG specialists as part of the nutrient criteria development process.

Case Study

Data from Minnesota provide an example of how this technique might be applied to help determine appropriate reference conditions for an ecoregion. Though Minnesota's P criteria, which were eventually developed, were not established using the specific technique described in this text, it is instructive to see how the percentiles compare between a reference population and a random or preexisting database (as was the case in Minnesota). The table below displays phosphorus concentration at the 75th percentile for a distribution of reference lakes and 25th percentile for the distribution of total assessed lakes for three ecoregions.

In the NLF, the 75th percentile of the reference lakes was slightly higher than the 25th percentile for the random population (note that the random population would include reference lakes as well in this instance). The P criteria for that region was set just above the 75th percentile for the reference lakes. In the NCHF ecoregion, the 75th percentile of the reference lakes was again higher than the 25th percentile for the random population. The P criteria for this region fell between these two values. In the highly agricultural WCBP the 75th percentile of the reference lakes was well above the random data set; however, the random data set is rather small and there is substantial overlap between the two data sets (i.e., 12 of 45 lakes in the random data set were reference lakes. In the case the P criteria was set below both values but was relatively close to the 25th percentile for the random data set. This analysis suggests that this technique may merit attention in those regions where there are both reference and larger random or preexisting data sets for the type of analysis.

Finding Reference Condition Values for Phosphorus ($\mu g/L$) Concentration Based on Reference and Total Assessed Distributions by Ecoregion in Minnesota

Ecoregion	75th Percentile of Reference Lakes	25th Percentile of Total Assessed Lakes	P Criteria for Swimmable Use
Northern lakes and forests	27 (n=30)	16 (n=543)	30
North central hardwood forest	50 (n=38)	35 (n=368)	40
Western corn belt plains	150 (n=12)	97 (n=45)	90